REMARKS

Status of the Claims

Claims 6-9 are pending in the application. Claims 6-9 are rejected. Claim 6 is amended herein. Claim 7 is canceled. Claims 8-9 are not amended.

Claim Amendments

Claim 6 is amended to incorporate the limitation of canceled claim 7 and to clarify the method steps in response to advisory action mailed on August 23, 2004.

Amended claim 6 recites a method of measuring the amount of oxidative stress in an individual. This method comprises the steps of collecting tissue of interest from the individual. The presence of oxidative stress in the tissue of interest is determined by measuring an amount of mitochondrial DNA damage per length of mitochondrial DNA and determining a decrease in at least mRNA production and one or more of protein production, oxidative phosphorylation and ATP production in the tissue of interest. Further, an amount of DNA damage per length of DNA in a nuclear gene in the tissue of interest is also measured where the nuclear gene is selected from the group consisting of the β-globin locus, transcriptionally active genes and transcriptionally inactive genes. Subsequently, the mitochondrial DNA damage is compared with the DNA damage in the nuclear gene, where a greater amount of DNA damage per length of mitochondrial DNA than per length of nuclear DNA is indicative of oxidative stress.

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In the advisory action, the Examiner states that the claim amendments in the response to the Final Office Action were not entered since the newly amended claim raised a 35 U.S.C. Section 112/2nd paragraph issue. According to the Examiner, since the newly amended claim had 2 steps numbered (c), it was not clear whether measuring one or more of mRNA production, protein production... was in addition to measuring the amount of mitochondrial DNA or whether this was the means that the mitochondrial DNA was measured. Further, due to the amendment it was also unclear how step d now added to the claims since the first step c provided the correlation.

Applicants have since amended claim 6 further as discussed above to clarify the points raised by the Examiner. The newly amended claim 6 is directed to a method of measuring the amount of oxidative stress in an individual that are supported by the teachings of the specification. After collecting the tissue of interest from the individual, the amount of mitochondrial DNA damage per length of mitochondrial DNA is measured. Additionally, a decrease in at least mRNA production, and one or more of protein production, oxidative phosphorylation and ATP production is determined. The combination of these two parameters will determine the presence of oxidative stress in the individual. By demonstrating a link between oxidative mitochondrial DNA damage, altered gene expression and mitochondrial dysfunction *in vitro* (Example 9, page 40-48), the instant invention supports this method step. Although this method step enables determination of oxidative stress, it does not measure the amount of oxidative stress as stated in the preamble of the claim. The specification clearly

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teaches measuring DNA damage in nuclear gene as recited In step (c) of amended claim 6 and the quantification of oxidative stress by comparing the mitochondrial DNA damage with the nuclear DNA damage (*in vitro* and *in vivo*) as recited in step (d) of amended claim 6. Hence in view of these amendments, Applicants submit that the claim amendments clarify all the points raised by the Examiner. Accordingly, Applicants respectfully request the Examiner to enter the proposed claim amendments.

Additionally, Applicants respond to the 35 U.S.C. 103(a), obviousness rejections of Final Office Action, mailed June 3, 2004 below.

Claims 6-9 were rejected under 35 U.S.C. 103(a) as being unpatentable over **Yan** *et al* (Circulation, 96(8): Suppl. P. I605, (1997), referred to as **Yan**) in view of either **Corral-Debrinski** *et al* (Mutation Research, 275: 169-180 (1992), referred to as **Corral-Debrinski I**) or **Corral-Debrinski** *et al* (JAMA, 266(13): 1812-1816 (1991), referred to as **Corral-Debrinski II**)

The Examiner found the Applicants' response to previous office action unpersuasive and had maintained rejections of previous office action since some of these arguments were directed to individual references and not combination of all references. Additionally, the Examiner maintained that **Coral-Debrinski II** taught that all of the hearts with ischemia due to atherosclerosis had much higher levels of mitochondrial DNA (mtDNA) deletion, which was consistent with the claim of instant invention. Therefore, the Examiner contended that the teachings of **Coral-Debrinski I and II** along with the teachings of **Yan** provide art

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recognized means for detection of DNA damage. Applicants respectfully traverse this rejection.

Applicants have already discussed above how the teachings of the specification support all the elements in the amended claim 6, in addition to clarifying the points raised by the Examiner in the Advisory Action. Additionally, the instant invention also provides the first evidence for a role of mitochondrial dysfunction in atherogenesis by teaching that: (1) mitochondrial DNA damage is significantly increased in human and mouse atherosclerotic aorta; (2) aortic mitochondrial DNA damage increases with age *in vivo*; (3) mitochondrial DNA damage occurs *in vivo*; (3) mitochondrial DNA damage occurs prior to or is coincident with atherosclerotic lesion development *in vivo*; (4) measurement of mitochondrial DNA at an early stage provides an accurate assessment of reactive species and oxidative damage-mediated atherosclerotic risk; and (5) the putative role of SOD2 in preventing atherogenesis in areas of turbulent flow may involve protection of the mitochondrial genome form oxidative damage (Example 20).

The Applicants contend that the combined teachings of **Yan**, **Corral-Debrinski I** and **II** do not teach all the elements of the newly amended claim 6. As discussed earlier, the instant invention teaches of an increase in mitochondrial DNA damage and a decrease in the mitochondrial DNA encoded gene transcripts (ND2 and cytochrome b), protein production, oxidative phosphorylation and ATP production. **Yan** neither teaches measuring these parameters nor does it suggest correlating them with mitochondrial DNA damage. Although both **Corral-Debrinski I** and **II** teach of measuring OXPHOS gene transcripts, **Corral-**

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Debrinski II teaches of increase in the mitochondrial DNA transcript for cytochrome b in ischemic hearts (table 3). Additionally, **Corral-Debrinski II** teaches that since analysis of hearts that failed for a variety of other reasons demonstrated increased OXPHOS gene expression irrespective of their levels of mitochondrial DNA damage, the OXPHOS gene induction may be part of general response to chronic cardiac failure (page 1815, col. 3, last para.). Thus, **Corral-Debrinski II** teaches away from the instant invention. Therefore, if one with ordinary skill in the art were motivated to measure the amount of oxidative stress based on the combined teachings of the three cited references, one would either not correlate a decrease in mitochondrial mRNA production with mitochondrial DNA damage or one would correlate increased mitochondrial mRNA production with mitochondrial DNA damage. Neither of these would enable one of ordinary skill in the art to arrive at the instant invention.

Applicants assert that obviousness requires that the prior art relied upon fairly teach or suggest all the elements of the instant invention and that an incentive or motivation be present in the prior art to produce the claimed invention with reasonable expectation of success in its production. The Applicants have shown that the combined teachings of **Yan, Corral-Debrinski I and II** do not teach or suggest all the elements of the present invention, nor do they provide an incentive or motivation to produce the claimed invention with reasonable expectation of success in its production. Hence, the subject matter of the present invention is not obvious to one with ordinary skill in the art at the time the invention was made. Additionally claims 7 and 9 are dependent on claim 6, which

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cannot be rendered obvious by **Yan, Corral-Debrinski I and II**. Accordingly, based on the above-mentioned remarks and amendments, the Applicants respectfully request that the rejection of claims 6, 7 and 9 under 35 U.S.C. 103(a) be withdrawn.

Additionally, Claim 8 was rejected under 35 U.S.C. 103(a) as being unpatentable over **Yan et al.** (Circulation, 96(8): Suppl. P. I605, (1997), referred to as **Yan**) in view of either **Corral-Debrinski et al.** (Mutation Research, 275: 169-180 (1992), referred to as **Corral-Debrinski I**) or **Corral-Debrinski et al.** (JAMA, 266(13): 1812-1816 (1991), referred to as **Corral-Debrinski II**) and further in view of **Van Houten** (U.S. Pat. 5,989,816 (1999)).

The Examiner had stated that although **Yan, Corral-Debrinski I** or **II** did not specifically teach treating DNA with FAPY glycosylase prior to PCR amplification as specified in claim 8, **Van Houten** taught the method for detection of DNA damage by detecting 8-oxo-deoxyguanosine (8-oxo-G-lesion) using FAPY glycosylase. Specifically, **Van Houten** taught that the assay efficiently detects most forms of base damage and DNA single and double strand breaks. Further, **Van Houten** taught that FAPY converts the 8-oxo-dG strand break with a glycosylase/endonuclease from E.coli and the DNA was used to determine the number of lesions/17.7kb. Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the mitochondrial DNA damage methods of **Yan** in view of **Corral-Debrinski I** or **II** with the teachings of **Van Houten**. Applicants respectfully traverse this rejection.

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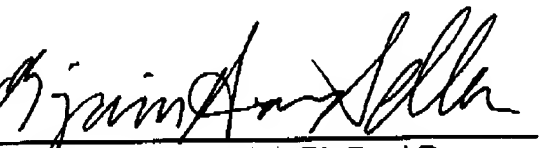
Claim 8 depends on amended claim 6. Applicants maintain that since the combined teachings of **Yan**, **Corral-Debrinski I** and **II** do not render claim 6 obvious, they cannot render claim 8 obvious either. Therefore, although **Van Houten** teaches the detection of DNA damage by detecting 8-oxo-deoxyguanosine using FAPY glycosylase, the combined teachings of **Van Houten** and the above cited references do not render claim 8 obvious. Accordingly, based on the above-mentioned remarks, the Applicants respectfully request that the rejection of claim 8 under 35 U.S.C. 103(a) be withdrawn.

This is intended to be a complete response to the Advisory Action, mailed August 23, 2004 and Supplemental to the Final Office Action, mailed June 3, 2004. Applicants submit that the pending claims are in condition for allowance. If any issues remain outstanding, please telephone the undersigned attorney of record for immediate resolution.

Respectfully submitted,

Date: 9/13/04



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